TITLE

MLA-3202, Batch RC-1045, CAS 1454803-04-3
SKIN SENSITIZATION IN GUINEA PIGS

TEST GUIDELINE

OCSPP 870.2600 and OECD 406

AUTHOR

Vincent A. Murphy, PhD, DABT

STUDY COMPLETION DATE

10 November 2016

PERFORMING LABORATORY

STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY ID

20252-16

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The following is a detailed description of all differences between practices used in the study and those required by EPA TSCA 40 CFR Part 792; OECD ENV/MC/CHEM (98) 17.
Section 792.113 (a) -- Section II, 6.2 (5): mixture analysis was not performed.
Section 792.31 (d) and 792.105 (a) -- Section II, 1.1 (2)(p), 6.1 (1) and 6.2 (2): the provided Certificate of Analysis was not accompanied by a GLP compliance statement.
Section 792.31 (d) and 792.105 (b)(e) -- Section II, 6.2 (4): certified stability information was not provided to testing facility.

Study Director: Vincent A. Murphy Date: 10 Nov 16
Vincent A. Murphy, PhD, DABT
STILLMEADOW, Inc.

Sponsor:_____ Date:_____
Name of Signer:_____
Sponsor: Chemtura

Submitter:_____ Date:_____
Name of Signer:_____
Submitter: Chemtura

QUALITY ASSURANCE STATEMENT

Study Title: MLA-3202, Batch RC-1045, CAS 1454803-04-3
Skin Sensitization in Guinea Pigs

The study report and data have been audited in accordance with Good Laboratory Practice standards and STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The final report accurately reflects study data. The Quality Assurance Unit has not been involved in the actual conduct of this study.

The Quality Assurance Unit performed a facility inspection on 22 Jul 16. All findings were reported to Management, and the report and responses are kept in Quality Assurance files.

Findings from any study inspections and audits were reported to the Study Director and Management as follows:

Critical Phase Inspected	Date Inspected	Reported to Study Director	Reported to Management
Protocol Review	16 Aug 16	16 Aug 16	16 Aug 16
Test Substance Dispense/Dose Prep Observations	14 Sep 16 14 Oct 16	14 Sep 16 14 Oct 16	14 Sep 16 14 Oct 16
Report/Data Audit	31 Oct 16	1 Nov 16	1 Nov 16

K Argueta — 01Nov16

Kelia Argueta, BS
Quality Assurance, STILLMEADOW, Inc.

Date

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SUMMARY

A skin sensitization study was conducted on 15 male and 15 female short-haired albino guinea pigs to determine if test substance MLA-3202, Batch RC-1045, CAS 1454803-04-3 produced a sensitizing reaction. Males and females were assigned to each of two groups, designated Naive control (5/sex) and Test (10/sex). Naive control group animals remained untreated during induction phase of the study. Test group animals were treated with 0.4 mL of undiluted test substance (selected from range-finding). Test animals were treated once weekly for three weeks, for a total of three inductions. After a two-week rest period, all animals (both groups) were challenged at a virgin test site with an application of 0.4 mL of undiluted test substance.

The test substance produced no reaction in either Test animals or Naive control animals after the challenge treatment. Therefore, MLA-3202, Batch RC-1045, CAS 1454803-04-3 is not a sensitizer in guinea pigs.

INTRODUCTION

The study objective was to determine sensitizing potential of the test substance using a modification of the Buehler method (Ritz, HL, and Buehler, EV, "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests", Current Concepts in Cutaneous Toxicity, p. 25-42, Academic Press, NY, 1980). This study was conducted in accordance with US EPA OCSPP 870.2600 and OECD 406 guidelines, approved protocol (Appendix A) and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol that affected study quality or outcome. All procedures used in this study are in compliance with Animal Welfare Act Regulations. The protocol, raw data, this report and a test substance sample are archived at STILLMEADOW, Inc. The study was initiated on 25 Aug 16, and the pre-dose experimental portion began on 5 Sep 16. Animals were treated as follows, and the study terminated on 14 Oct 16.

Group	Induction Treatments			Challenge Treatment
	First	Middle	Last	
I Naive Control	--	--	--	12 Oct 16
II Test	14 Sep 16	21 Sep 16	28 Sep 16	12 Oct 16

SPONSOR INFORMATION

Company Name:	Chemtura
Address:	199 Benson Road Middlebury, CT 06749

TEST SUBSTANCE

Reference Name:	MLA-3202, Batch RC-1045, CAS 1454803-04-3
Label Identification:	MLA-3202, Lot RC-1045 This sample is intended solely for research and development purposes under the supervision of a technically qualified individual.
Date & Quantity Received:	25 August 16, 163.7 g (GW)
Physical Description:	Clear amber-red liquid
Storage:	Room temperature
Purity:	Refer to Certificate of Analysis (Appendix B)
Stability:	Exp: 17 Feb 19 per label information
Concentrations Administered:	Induction: 100% as received Challenge: 100% as received

Data generated for characterization and stability are the responsibility of the Sponsor. Records pertaining to identity, synthesis methods and location of documentation are the responsibility of the Sponsor.

TEST SYSTEM

Experimental Animals

Species & Strain:	Guinea Pig; Hartley-Albino
Justification of Species:	The guinea pig is conventionally used in skin sensitization studies to provide information on which human hazard can be judged, and is preferred by regulatory agencies.
Source:	Charles River, Hdq: Wilmington, MA
Quantity & Sex:	2/sex (Range-finding); 15/sex (Definitive); females nulliparous & non-pregnant
Acclimation Period:	At least 5 days
Date Born/Date Received:	17 & 22 Jun 16 / 22 Aug 16
Animal Identification:	Ear punch & cage card
Weights When Tested:	388 - 563 g

Animal Husbandry

Cage Type:	Stainless steel, suspended, wire bottom with plastic liner & bedding
Housing:	1 - 5 per cage (males separate from females); enrichment provided
Environmental Controls Set to Maintain:	· 20 ± 3°C target temperature · 30 - 70% target humidity · 12-hr light/12-hr dark cycle · 10+ air changes per hour
Actual Temp/Rel. Humidity:	16 - 25°C / 50 - 79%
Food:	LabDiet® 5025 Guinea Pig Diet; available ad libitum
Water:	Municipal water supply analyzed by TCEQ Water Utilities Division; available ad libitum from water bowls

Animal husbandry and housing at STILLMEADOW, Inc. comply with standards outlined in "Guide for the Care and Use of Laboratory Animals" (NRC Publ.). No contaminants were expected to have been present in feed or water that would have interfered with or affected study results.

POSITIVE CONTROL INFORMATION

Positive Control Material

Alpha-Hexylcinnamaldehyde, >95%, CAS # 101-86-0, Lot: MKBS2347V, Mfg: Aldrich
Concentrations Administered: Induction & Challenge: 0.4 mL

Positive Control Testing

Sensitivity of guinea pigs to a positive control material is confirmed in this laboratory periodically. The positive control animals used to conduct this study were supplied by Charles River Laboratories, and were tested according to the Buehler method.

STILLMEADOW, Inc. Study No. 20014-16
In-life started: 27 Apr 16; In-life completed: 27 May 16

Results: Data from this study are presented as Positive Control Tables (1 and 2). The mean score of 0.8 for the Test group after challenge treatment, when compared with the Naive control group mean score of 0.0, confirmed sensitivity of guinea pigs to the positive control material.

PROCEDURES

Irritation Range-Finding

Two male and two female albino guinea pigs were selected for irritation range-finding (Diagram 1) to determine both maximum dose producing no more than moderate irritation, and maximum non-irritating dose. Concentrations tested in the range-finder were 100% (undiluted), and 75%, 50% and 25% v/v dilutions in DI water, with each animal receiving 0.4 mL of each concentration at different test sites.

Preparation of Animals

Males and females were selected for each of two treatment groups, designated as Naive control group (5/sex) and Test group (10/sex). On the day prior to each treatment, animals were prepared by clipping the back of the trunk free of hair to expose a sufficiently sized test area on each animal. Individual body weights were recorded on Days 0 and 31.

Test Substance Administration

Based on range-finding results, the dose administered was an application of 0.4 mL of undiluted test substance. For each induction treatment, Test group animals were treated by introducing test substance beneath ~5 x 5 cm surgical gauze patch. Each gauze patch was placed laterally from the midline of the back on the left front quadrant of exposure area (Diagram 2), held by a strip of clear polyethylene film placed over the patch and secured with non-irritating tape. At the end of the 6-hour exposure period, wrappings and patches were removed, and animals returned to their cages. Test animals were treated once weekly for three weeks (Days 1, 8 and 15) with 0.4 mL of undiluted test substance, using the same treatment regimen and test site location for all three inductions. Naive group animals remained untreated during induction phase of the study.

Challenge Treatment

After a two-week rest period, all animals (both groups) were each challenged on Day 29 at a virgin test site with an application of 0.4 mL of undiluted test substance. The dose was applied in a manner identical to induction treatments, except the test site was placed laterally on the right rear quadrant of exposure area (Diagram 2).

Observations and Scoring Method

Observations for skin reactions at each test site were made ~24 hours after each unwrap. In addition, observations for skin reactions were made ~48 hours after both the first induction and the challenge unwraps. The scoring scale for grading skin reactions is presented as follows.

Erythema	Score
No reaction	0
Very faint, usually nonconfluent	0.5
Faint, usually confluent	1
Moderate	2
Strong, with or without edema	3

An average score for each time period was obtained by adding all scores for each time period and dividing by number of test sites scored for that time period. The test substance is considered a sensitizer if mean irritation scores, total number of animals with scores, and/or total number of scores for the virgin test site in the Test group after challenge treatment are appreciably greater than those for the Naive challenge group.

RESULTS AND DISCUSSION

Protocol Deviations

Temperature and relative humidity were at times outside protocol range; six animal weights were above protocol range. The deviation(s) did not affect study outcome.

Evaluation

Irritation range-finding results are presented in Diagram 1. Test site locations are presented in Diagram 2. Skin reactions and average skin reaction scores during induction and challenge are presented in Tables 1 and 2, respectively. Individual body weights are presented in Table 3. Average skin reaction scores for each group at challenge are as follows.

Group	Mean Challenge Scores
Naive Control	0.0
Test	0.0

CONCLUSION

The test substance produced no reaction in either Test animals or Naive control animals after the challenge treatment. Therefore, MLA-3202, Batch RC-1045, CAS 1454803-04-3 is not a sensitizer in guinea pigs.

Study Director: Vincent A. Murphy 10 Nov 16

Vincent A. Murphy, PhD, DABT
Director of Toxicology, STILLMEADOW, Inc.

Date

STUDY PERSONNEL

Technical Staff: Paul Siemens, BA
Charlie Mollo
Sean Taylor
Hector Fuentes
Mariana Cortez, AAS
Maurice Dufilho IV

Data Services: Connie Pavatte, Report Typist

DIAGRAMS
Test Substance: MLA-3202, Batch RC-1045, CAS 1454803-04-3
Skin Sensitization in Guinea Pigs

Diagram 1 - Range-finder
Date of Dosing: 6 Sep 16
Note: A dose of 0.4 mL/site was used. Each dilution was v/v in DI water.

31-M	
1	2
3	4

32-M	
4	1
2	3

33-F	
3	4
1	2

34-F	
2	3
4	1

1 = 100% 2 = 75% 3 = 50% 4 = 25%

Animal Number	Body Wt (g)	Observation Time After Treatment							
		24 Hour Score*				48 Hour Score*			
		Front Site		Rear Site		Front Site		Rear Site	
31-M	415	0	0	0	0	0	0	0	0
32-M	420	0	0	0	0	0	0	0	0
33-F	405	0	0	0	0	0	0	0	0
34-F	398	0	0	0	0	0	0	0	0

* - Observations made for erythema; M - Male; F - Female

Level Selected for Induction: 100% as received
Level Selected for Challenge: 100% as received

Diagram 2 - Test Site Location

HEAD OF ANIMAL	
LF	
	RR

LF - Left Front Test Site; RR - Right Rear Test Site

TABLE 1 - Skin Reaction Scores
Test Substance: MLA-3202, Batch RC-1045, CAS 1454803-04-3
Skin Sensitization in Guinea Pigs

Animal No.	Hours After Day of Treatment					
	Induction Treatments LF				Challenge RR	
	Day 1		Day 8	Day 15	Day 29	
	24 hr	48 hr	24 hr	24 hr	24 hr	48 hr
Naive Control						
41-M					0	0
42-M					0	0
43-M					0	0
44-M					0	0
45-M					0	0
46-F					0	0
47-F					0	0
48-F					0	0
49-F					0	0
50-F					0	0
Test						
51-M	0	0	0	0	0	0
52-M	0	0	0	0	0	0
53-M	0	0	0	0	0	0
54-M	0	0	0	0	0	0
55-M	0	0	0	0	0	0
56-F	0	0	0	0	0	0
57-F	0	0	0	0	0	0
58-F	0	0	0	0	0	0
59-F	0	0	0	0	0	0
60-F	0	0	0	0	0	0
61-M	0	0	0	0	0	0
62-M	0	0	0	0	0	0
63-M	0	0	0	0	0	0
64-M	0	0	0	0	0	0
65-M	0	0	0	0	0	0
66-F	0	0	0	0	0	0
67-F	0	0	0	0	0	0
68-F	0	0	0	0	0	0
69-F	0	0	0	0	0	0
70-F	0	0	0	0	0	0

M – Male; F – Female
LF – Left Front test site; RR – Right Rear test site
Note: Observations were made for erythema

TABLE 2 - Average Skin Reaction Scores
Test Substance: MLA-3202, Batch RC-1045, CAS 1454803-04-3
Skin Sensitization in Guinea Pigs

	Hours After Day of Treatment					
	Induction Treatments LF				Challenge RR	
	Day 1		Day 8	Day 15	Day 29	
	24 hr	48 hr	24 hr	24 hr	24 hr	48 hr
Naive Control						
					0.0	0.0
Mean					0.0	
Test						
	0.0	0.0			0.0	0.0
Mean	0.0		0.0	0.0	0.0	

LF - Left Front test site; RR - Right Rear test site

TABLE 3 - Body Weights

Animal Number	Day of Study		Animal Number	Day of Study	
	Day 0	Day 31		Day 0	Day 31
Naive Control					
41-M	516	669	46-F	407	522
42-M	502	667	47-F	388	508
43-M	449	646	48-F	406	558
44-M	481	680	49-F	421	528
45-M	479	643	50-F	469	577
Test					
51-M	549	793	61-M	498	560
52-M	494	707	62-M	466	584
53-M	476	756	63-M	474	579
54-M	464	641	64-M	506	631
55-M	563	873	65-M	505	557
56-F	449	555	66-F	435	696
57-F	440	564	67-F	451	715
58-F	434	526	68-F	464	781
59-F	443	569	69-F	453	712
60-F	429	547	70-F	442	718

M – Male; F – Female
Note: Weights are in grams

POSITIVE CONTROL TABLES

Positive Control Material: Alpha-Hexylcinnamaldehyde, Study: 20014-16

Table 1 - Positive Control Skin Reaction Scores

Animal No.	Hours After Day of Treatment					
	Induction Treatments LF				Challenge RR	
	Day 1		Day 8	Day 15	Day 29	
	24 hr	48 hr	24 hr	24 hr	24 hr	48 hr
Naive Control						
41-M					0	0
42-M					0	0
43-M					0	0
44-M					0	0
45-M					0	0
46-F					0	0
47-F					0	0
48-F					0	0
49-F					0	0
50-F					0	0
Test						
51-M	0	0	0	0.5	2	1
52-M	0	0	0	0	0.5	0
53-M	0	0	0	0	0.5	0.5
54-M	0	0	0	0.5	1	0.5
55-M	0	0	0	0	1	1
56-F	0	0	0	0.5	0.5	0.5
57-F	0	0	0	0	1	0.5
58-F	0	0	0	0	1	1
59-F	0	0	0	0.5	0.5	0
60-F	0	0	0	1	1	1

M – Male; F -- Female; LF -- Left Front test site; RR – Right Rear test site

Note: Observations were made for erythema

Table 2 - Positive Control Average Skin Reaction Scores

	Hours After Day of Treatment					
	Induction Treatments LF				Challenge RR	
	Day 1		Day 8	Day 15	Day 29	
	24 hr	48 hr	24 hr	24 hr	24 hr	48 hr
Naive Control					0.0	0.0
Mean					0.0	
Test	0.0	0.0			0.9	0.6
Mean	0.0		0.0	0.3	0.8	

LF - Left Front test site; RR - Right Rear test site

APPENDIX A - Protocol

STILLMEADOW
INCORPORATED

PROTOCOL For STUDY 20252-16

Test Substance:	MLA-3202, Batch RC-1045, CAS 1454803-04-3
Study Title:	SKIN SENSITIZATION In GUINEA PIGS
Guideline:	OCSPP 870.2600 & OECD 406
Test Facility:	STILLMEADOW, Inc. 12852 Park One Drive Sugar Land, TX 77478

Approved: Vincent A. Murphy — 25 Aug 16
Vincent A. Murphy, PhD, DABT — Date
Study Director, STILLMEADOW, Inc.

Approved: Robert J. Sabol — 16 Aug 16
Robert J. Sabol — Date
President, STILLMEADOW, Inc.

Reviewed: Kristina Rodrigue — 16 Aug 16
Kristina Rodrigue, RQAP-GLP — Date
Quality Assurance Director, STILLMEADOW, Inc.

Sponsor: Chemtura
199 Benson Road
Middlebury, CT 06749
203-573-3855
audrey.batoon@chemtura.com

Approved: Audrey Batoon — 16 Aug 16
Audrey Batoon, PhD — Date
Senior Toxicologist

A. GENERAL

1.	Study Title:	Skin Sensitization in Guinea Pigs
2.	Purpose:	To determine skin sensitization potential of test substance in guinea pigs.
3.	Method Guidelines:	This study will be conducted according to US OCSPP 870.2600 and OECD 406.
4.	Regulatory Compliance:	This study will be conducted in compliance with Good Laboratory Practice (GLP) standards: 1. EPA TSCA 40 CFR 792 2. OECD ENV/MC/CHEM(98)17 In the event of a regulatory inspection, Regulatory Inspectors will be provided with all study documentation requested. Sponsor will be notified of inspection of their study. All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOP).
5.	Quality Assurance:	The Quality Assurance Unit (QAU) will review the protocol. Study information will be entered into the master schedule. In-progress inspection(s) will be performed to ensure integrity of the study. Any deviations from SOP, protocol or GLP standards will be reported to Study Director and Management. Raw data and report will be audited, and a statement prepared and signed which will specify dates inspections were made and findings reported to Management and Study Director.
6.	Test Substance:	MLA-3202, Batch RC-1045, CAS 1454803-04-3. Test substance identification should include name, lot/batch number and purity. Sponsor should also provide information regarding safety, storage conditions and disposal. Sponsor assumes responsibility for purity, stability, identity, synthesis methods and location of documentation.
7.	Positive Control Material:	Alpha-Hexylcinnamaldehyde (CAS # 101-86-0) or alternate known sensitizer
8.	Proposed Schedule:	Testing will begin within ~2 months of test substance receipt and authorization to conduct the study. Proposed Experimental Start & End: 31 Aug 16 - 30 Sep 16 Test portion: 31 days; extended if rechallenge is conducted
9.	Study Director:	Vincent A. Murphy, PhD, DABT
10.	Experimental Summary:	Skin of Test group guinea pigs will be treated once weekly for 3 weeks, for a total of three induction treatments, with maximum concentration of test substance that produced no more than moderate irritation (MIC) in range-finder. After a 2-week rest period, animals will be challenged with a final treatment to a virgin test site with maximum non-irritating concentration (MNIC) of test substance. Naive Control animals will remain untreated during the three inductions and receive same treatment as Test group animals for challenge. Skin irritation scores will be determined after each unwrap. Regimen is based on procedures outlined in Ritz, HL, and Buehler, EV, Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, Current Concepts in Cutaneous Toxicity, 1980, Academic Press. Test substance will be considered a sensitizer if mean irritation score, total number of animals with scores, and/or persistence of scores for the virgin test site in Test group after challenge treatment are appreciably greater than those for naive Control group.
11.	Protocol Amendments:	Any alteration in the protocol will be justified, approved by Study Director, and recorded in writing.
12.	Sponsor Audits:	Sponsor may send an authorized Representative to inspect test system and/or data on STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a. Species/Strain/Source:	Guinea Pig; Hartley Albino; Charles River, Hdq: Wilmington, MA (or other suitable supplier)
b. Species Justification:	The guinea pig is conventionally used in skin sensitization studies to provide information on which human hazard can be judged, and is preferred by regulatory agencies.
c. Quantity & Sex:	2/sex (Range-finding); all females nulliparous & non-pregnant; Definitive Test: 10/sex Test group; 5/sex naive Control group 2/sex additional New-naive controls (if rechallenge required)
d. Age/Weight on Day 0:	Young adult; 340 - 500 g
e. Animal/Group ID:	Ear punch / Cage card
f. Acclimation & Health Status:	Animals will be acclimated for at least 5 days prior to initial dosing. Range-finding may be conducted during acclimation period. Normal weight gain, appearance and behavior will be factors used to select healthy naive animals for testing.

2. Animal Husbandry

a. Cage Type:	Stainless steel, suspended, wire bottom with plastic liner & bedding
b. No/Cage:	1 - 5, males separate from females (nulliparous & non-pregnant)
c. Enrichment:	Provided to all animals during study
d. Food:	LabDiet® 5025 Guinea Pig Diet or equivalent, available ad libitum; analyzed by manufacturer for nutritional content
e. Water:	Tap water, available ad libitum, water bowl or automatic system; municipal water supply analyzed by TCEQ Water Utilities Division
f. Contaminants:	There are no known contaminants in feed or water available to laboratory animals that would be expected to interfere with this study.
g. Environment:	Target temperature: 20° ± 3°C Target relative humidity: 30 - 70% 12-hour light/12-hour dark cycle (regulated automatically) Room ventilation: at least 10 air changes per hour

3. Dose Range-finding: Prior to definitive portion of the study, a range-finder with at least four concentrations of test substance will be conducted with 4 animals (2/sex) (Legend A). Range-finder will determine MIC upon initial dosing, to be used for the induction treatments. Range-finder will also determine MNIC (producing in 4 guinea pigs no more than two scores of 0.5 and two scores of 0.0 at the 24-hour reading) to be used for challenge. Skin reactions observed after first induction treatment, if different from expected based on range-finder, may also be considered when challenge concentration is selected.

Concentrations for liquid test substances typically will be 100% undiluted, and 75%, 50% and 25% v/v dilutions in DI water (unless solubility characteristics require another solvent, such as 80% ethanol). A volume of 0.4 mL will be tested at each concentration.

B. 3. (cont.) Concentrations for solid test substances typically will be 400 mg undiluted (moistened with DI water), and 75%, 50% and 25% w/v dilutions in DI water. Amounts used will be 0.4 mL of each dilution. Another solvent may be used if solubility characteristics of test substance so require.

4. Test Substance Administration

a. Animal Preparation: Animals will be prepared on the day prior to each treatment by clipping exposure area on the back and flanks with animal clippers. Clipping will be repeated as necessary.

b. Route of Administration: Application of a topical patch will be employed.

c. Reason for Route of Administration: Dermal contact is a potential route of human exposure.

d. Test Substance Application: The first application will be on Day 1. Under ~5 x 5 cm gauze patch, 400 mg (solid) or 0.4 mL (liquid) test substance or dilution (MIC) will be applied to one of four possible test sites (right or left anterior, or right or left posterior). Gauze will be secured with a strip of clear polyethylene film and non-irritating adhesive tape. Animals may be placed in restrainers for duration of the 6-hour exposure period. Animals will then be removed from restrainers (if used), and wrappings removed. If residual test substance is present, test sites will be washed with room temperature tap water or appropriate solvent and dried. Each successive induction application will be made on the same site. At the first observation, if any, of strong irritation causing/expected to cause pain/distress, a sufficient concentration of suitable analgesic will be administered by subcutaneous or IM injection. Until irritation lessens, analgesic will be readministered at an appropriate frequency.

After a 2-week rest period, a virgin test site will be used for challenge treatment (MNIC).

e. Controls: A naive Control group will remain untreated during the three inductions and receive same treatment as Test group for the challenge. To confirm sensitization potential of guinea pig strain and validate procedure used, a Positive Control group will be tested (a separate STILLMEADOW, Inc. study) with a known sensitizer within ~6 months of this study period.

f. Dosing Schedule: 1) Induction: Animals in Test group will be dosed with MIC of test substance (produced no more than moderate irritation in range-finding) once weekly for 3 weeks (Day 1, 8 and 15). Animals will not be treated during the succeeding 2-week period.

2) Challenge: After this rest period, Test animals and naive Control animals will be challenged with MNIC of test substance at a previously untreated virgin test site on Day 29. Liquid test substances previously diluted in 80% ethanol will be diluted in acetone.

5. Observations

a. Body Weights: Body weights will be recorded for each animal on Days 0 and 31, and on Day 38 if rechallenged, or time of discovery if found dead during study.

b. Observations: Exposure sites will be scored for erythema with or without edema at ~24 hours after each unwrap (also at ~48 hours after initial induction and challenge) according to Buehler method (Legend A). Other signs of dermal irritation may also be noted. Animals that have damaged skin producing undue stress/discomfort will be humanely sacrificed after consulting Sponsor; each animal will be euthanized following final observation.

B. 6. Evaluation of Results:

Study Director will make a judgment of dermal sensitization after examining and comparing data for the challenge of Test group animals with naive Control animals. Judgment will be based on the following factors:

1) Mean skin irritation scores
2) Total number of animals with scores
3) Persistence of scores

Information provided will include the proportion that became sensitized and extent (faint, moderate, strong) of sensitization reaction in individual animals. If results are not conclusive, Study Director will consult with Sponsor regarding rechallenge exposures.

7. Rechallenge:

Rechallenge is indicated if all following conditions apply:

1) Induction treatments produced irritation,
2) Challenge was not conducted at 100% concentration, &
3) No clear difference between challenge responses of naive Control animals & Test animals

However, if only very slight irritation is observed in a few animals of both groups, substance is not a sensitizer and no rechallenge is needed.

If rechallenge is applicable, a 1-week rest period will follow initial challenge treatment. Animals will be rechallenged on Day 36 on a previously untreated test site with a higher concentration of test substance than used at challenge. At least 4 New-naive animals will be added to serve as irritation controls for the rechallenge. Study Director will then make a judgment based on both rechallenge and initial challenge data.

8. Test Substance Accountability:

A comprehensive inventory of test substance received and used will be kept. Test substance container(s) will be weighed when received at this facility, and all test substance use will be recorded. Test substance and substance dosing solutions will be stored in the original containers or equivalent, or in glass containers with Teflon-lined caps.

9. Disposal of Unused Test Substance:

Unused test substance will be disposed of at Sponsor's expense after termination of the study. A reserve sample will be retained by STILLMEADOW, Inc. for at least 5 years.

10. Safety Precautions:

General safety precautions required by laboratory SOPs will be followed. Sponsor will supply basic toxicity data on test substance to be used; however, since toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. Sponsor or Representative shall be notified of any exposures requiring a physician's examination or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and archived at STILLMEADOW, Inc. upon study termination:

a. Protocol & protocol amendments (if any)
b. Final report & amendments (if any)
c. Study correspondence
d. Animal receipt/acclimation data
e. Test substance receipt, identification as supplied by Sponsor, preparation, administration, disposition; data on vehicles used
f. Test animal information: number, species, strain, age, source, sex
g. Body weight data
h. Individual dermal irritation scores at ~24 hours (& 48 hours when applicable) after each unwrap (range-finding & definitive test)
i. Data from recent STILLMEADOW, Inc. Positive Control Study in its own study number file
j. Other pertinent data

C. 2. Data Storage: All raw data, and originals of both protocol and final report will be archived at STILLMEADOW, Inc. for at least 5 years.

3. Data Reporting: Final report will include following data as described in GLP standards:
a. Statement from QAU
b. GLP Compliance Statement & signature of Study Director
c. Names of scientific personnel involved in study
d. Dates of study initiation & termination
e. Identification, label information, description, storage of test substance, & identification of vehicles used
f. All pertinent animal data & husbandry, dosing information, observation methods
g. Reference to range-finding
h. Description of test procedures
i. Individual observations for erythema & any other defects at ~24 hours (& 48 hours when applicable) after each unwrap
j. Determination if test substance was/was not a sensitizer
k. Mean & individual skin irritation scores for each group for each time period
l. Individual body weight data
m. Results from recent STILLMEADOW, Inc. Positive Control Study
n. Copy of this protocol
o. Any protocol deviations & impact, if any, on study

4. Report Submission: A report will be submitted within ~6 weeks after termination of in-life portion of the study; if a draft report is issued, there will be a 90-day Sponsor approval period. Draft will be finalized upon Sponsor approval or at end of 90-day period.

LEGEND A

Diagram of Test Sites & Typical Doses/Concentrations for Range-Finding:

Animal 1	
1	2
3	4

Animal 2	
4	1
2	3

Animal 3	
3	4
1	2

Animal 4	
2	3
4	1

Liquid Test Substance Key - 1 = 100% 2 = 75% v/v 3 = 50% v/v 4 = 25% v/v

Solid Test Substance Key - 1 = 400 mg 2 = 75% w/v 3 = 50% w/v 4 = 25% w/v

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Buehler Sensitization Scoring Scale:

Erythema	Score
No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1
Moderate erythema	2
Strong erythema, with or without edema	3

APPENDIX B – Certificate of Analysis

Chemtura Corporation
12 Spencer St
Naugatuck, CT 06770

Analytical Services
www.chemtura.com

Certificate of Purity

Customer: Support for Toxicology Studies

Test Substance Name: MLA3202; Amides, tallow, N,N-bis(2-hydroxypropyl)

Physical Appearance: Liquid

CAS No.: 1454803-04-3

Ref. or Lot Number: RC-1045

Date of Analysis: revised March 18, 2016 (original issue March 7, 2016)

Percent Composition	Monoisotopic Mass (daltons)	Formula	Structure/ identity
33.1	397.4	$C_{24}H_{47}NO_3$	C18:1 (oleic) tallow amides, N,N-bis(2-hydroxypropyl)
22.9	371.3	$C_{22}H_{45}NO_3$	C16:0 (palmitic) tallow amides, N,N-bis(2-hydroxypropyl)
13.6	395.4	$C_{24}H_{45}NO_3$	C18:2 (linoleic) tallow amides, N,N-bis(2-hydroxypropyl)
11.0	399.4	$C_{24}H_{49}NO_3$	C18:0 (stearic) tallow amides, N,N-bis(2-hydroxypropyl)
6.0	369.3	$C_{22}H_{43}NO_3$	C16:1 (palmitoleic) tallow amides, N,N-bis(2-hydroxypropyl)
3.2	419.3	$C_{26}H_{45}NO_3$	C20:4 (eicosatetraenoic) tallow amides, N,N-bis (2-hydroxypropyl)
2.0	393.3	$C_{24}H_{43}NO_3$	C18:3 (linolenic) tallow amides, N,N-bis(2-hydroxypropyl)
1.5	282.3	$C_{18}H_{34}O_2$	C18:1 (oleic) acid
1.1	421.4	$C_{26}H_{47}NO_3$	C20:3 (eicosatrienoic) tallow amides, N,N-bis (2-hydroxypropyl)
5.6			Sum of residual components (< 1% each)
100.0			Total

Blake Lewis 3/7/16
Blake Lewis Date
Analytical REACh Scientist, Analytical Services

Colin Moore
Sign ABN 3/7/16
Albert J. Nitowski Date
Sr. Technology Manager
Analytical and Lab Support Services